PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 October 2000 (13.10.00)	
International application No. PCT/AU00/00181	**Applicant's or agent's file reference** 92368
International filing date (day/month/year) 10 March 2000 (10.03.00)	**Priority date** (day/month/year) 10 March 1999 (10.03.99)
Applicant DALL, David, James	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 August 2000 (30.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Manu Berrod
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

AU0000181

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00284

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] C12N 7/01, 15/79, 15/62, A01N 63/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[5] C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU IPC[5] C12N 7/01, 15/79

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
WPAT KEYWORDS-ENTOMOPOXVIRUS, POLYHEDROSISVIRUS, POLYHEDROSIS (W) VIRUS, HETEROLOG
BIOT KEYWORDS-POLYHEDROSIS VIRUS, POLYHEROSIS (W) VIRUS, HETEROLOG, EPV, NPV.
CASM KEYWORDS-ENTOMOPOXVIRUS, POLYHEDROSISVIRUS, POLYHEDROSIS (W) VIRUS, HETEROLOG, EPV, NPV.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,A, 37516/91 (THE TEXAS A & M UNIVERSITY SYSTEM) 2 April 1992 (02.04.92)	1-45
P,X	AU,A, 16634/92 (UNIVERSITY OF FLORIDA) 3 September 1992 (03.09.92)	1-45
Y	AU,A, 82133/91 (SMITHKLINE BEECHAM CORPORATION) 9 January 1992 (09.01.92)	1-45
Y	JOURNAL OF GENERAL VIROLOGY (1990), 71,1525-1534 published 1990	1-45

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1993 (02.09.93)	13 SEP 1993 (13·09·93)

Nam and mailing address f the ISA/AU

AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA

Facsimile No. 06 2853929

Authorized officer

J.W. ASHMAN

T lephon No. (06) 2832364

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	AU,A, 86550/91 (THE TEXAS A & M UNIVERSITY SYSTEM) 2 April 1992 (02.04.92)	
A	AU,A, 54780/90 (YUEN K L et al.) 15 November 1990 (15.11.90)	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 92368	**FOR FURTHER ACTION** ·	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU00/00181**	International Filing Date *(day/month/year)* 10 March 2000	Priority Date *(day/month/year)* 10 March 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] A01H 5/00; A01N 63/02

Applicant

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
30 August 2000	20 March 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **CHRISTOPHER LUTON** Telephone No. (02) 6283 2256

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/00181

I. Basis f the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-3, 5, 8-24,** as originally filed,
pages , filed with the demand,
pages **4, 6, 7,** received on **15 January 2001** with the letter of **15 January 2001**

☒ the claims, pages **25,** as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **26, 27,** received on **13 March 2001** with the letter of **13 March 2001**

☒ the drawings, pages **1/7-7/7,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1/8-8/8,** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☒ the claims, Nos. **22**

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to n velty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-21**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-21**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-21**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 - Mitsuhashi et al.

D2 - Tomita et al.

D3 - Xu and Hukuhara

D4 - Dall et al.

D5 - Xu and Hukuhara

D6 - Chemical Abstracts online abstract accession no. 125:295128

D7 - Hayakawa et al.

D8 - Gauthier et al.

D9 - WO 93/25666

NOVELTY (N) and INVENTIVE STEP (IS) Claims 1-21

The citations do not disclose or suggest plants transformed with spindle body or spindle-like body proteins. The citations do not disclose or suggest methods of controlling or preventing insect damage to plants by the application of spindle bodies or spindle-like bodies thereto. Hence, the claims would appear to be novel and to involve an inventive step in light of the citations.

promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

In a second aspect, the present invention provides a feed bait composition comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier, with the proviso that the feed bait composition does not further comprise a nuclear polyhedrosis virus.

Detailed disclosure of the Invention:

As mentioned above, the invention provides a plant capable of expressing one or more constituent SB/SLB protein(s) in tissues (e.g. leaf tissue or a product tissue such as fruit tissue) susceptible to damage by feeding insects. Thus, when feeding insects feed on a plant according to the invention, they will ingest, along with plant tissue, the expressed constituent SB/SLB protein(s). Since SBs/SLBs appear to inhibit feeding, growth and/or development of insects and, potentially, increase susceptibility to infection from insect pathogens (and thereby insect death), ingestion of one or more of the constituent SB/SLB protein(s) by feeding insects may reduce further damage to the plant. In addition, it is believed that inhibiting the feeding, growth and/or development of insects also increases the likelihood of insect death resulting from, for example, adverse environmental conditions, predators and chemical and other biological agents (e.g. pathogenic bacteria).

The plant according to the invention may be any plant of agricultural, arboricultural, horticultural or ornamental value that is susceptible to damage by feeding insects. Preferably, the plant is selected from plants of agricultural value such as cereals (e.g. wheat and barley), vegetable plants (e.g. tomato and potato) and fruit trees (e.g. citrus trees and apples). Other preferred plants include tobacco and cotton.

The polynucleotide molecule(s) comprising a nucleotide sequence encoding one or more constituent SB/SLB protein(s) operably linked to a suitable promoter sequence(s), may be any polynucleotide molecule(s) that may be stably segregated and retained in daughter cells. Preferably, the polynucleotide molecule(s) is stably integrated into a non-essential site within the plant genome (as may be achieved by the well known technique of homologous recombination).

mosaic virus (CaMV 35S) promoter element, and promoter elements from the sub-clover stunt virus (SCSV).

Plants according to the present invention may also express an exogenous toxin or other exogenous agent that is deleterious to insects. For example, the plant may also express a *Bacillus thuringiensis* δ-toxin, an insect neurohormone, or an antisense RNA or ribozyme targeted against an essential cellular function. The heterologous toxin or deleterious agent may be encoded by a nucleotide sequence (operably linked to a suitable promoter sequence) borne on the polynucleotide molecule(s) encoding the one or more constituent SB/SLB protein(s) or may be borne on a further polynucleotide molecule which has been co-transformed into the plant.

Transformation of the plant with the polynucleotide molecule(s) may be achieved by any of the methods well known in the art including *Agrobacterium* transformation and electroporation.

As will be appreciated, the benefits achieved by expressing one or more constituent SB/SLB protein(s) in plants might also be achieved by producing feed baits comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies. Thus, feed bait compositions according to the present invention comprise spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier, with the proviso that the feed bait compositions do not further comprise a nuclear polyhedrosis virus.

The feed bait compositions may be in a liquid or gel form, but more preferably are in a solid form. The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) may comprise 0.05 to 15.0% (by weight) of the composition. In addition to the spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) and the agriculturally acceptable carrier, the feed bait composition may further comprise a pheromone(s) or other chemical attractant to insects. For liquid formulations the agriculturally acceptable carrier may be selected from ingredients such as milled clays or edible carrier substances such as plant materials, molasses or raw sugar, and microorganisms such as yeasts or other fungi, algae or bacteria. For solid feed bait compositions, the agriculturally acceptable carrier may be selected from ground or fragmented plant material and other materials as described above

processed to an appropriate form. The solid feed bait compositions may be provided as pellets and applied by casting over an area containing a plant for which protection against damage by feeding insects is desired. Liquid or gelled feed bait compositions may be applied to a plant by spraying.

The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) included in the feed bait composition may be isolated from natural sources or, more conveniently, produced recombinantly in, for example, bacteria, yeast, insect or mammalian cell cultures.

Insects having ingested spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) as the result of having fed on a plant or feed bait composition according to the present invention may, as mentioned above, be expected to cause reduced damage to plants either as a result of reduced feeding/growth and/or reduced life times as a result of an increased susceptibility to adverse environmental conditions or chemical and biological agents. Accordingly, the present invention further extends to methods where a plant in accordance with the first aspect or a plant to which a feed bait composition in accordance with the second aspect has been applied, is treated with an insecticidal chemical and/or biological agent, and especially one whose activity has been shown to be higher against smaller, as compared to larger, insect larvae. Suitable chemical agents include organophosphate compounds and suitable biological agents include pathogenic bacteria (especially *Bacillus thuringiensis* [Bt]) and insect viruses other than nuclear polyhedrosis viruses. These agents may be applied by any of the methods well known in the art and, most conveniently, by spraying. Preferably, the chemical or biological agent is applied in the form of a composition comprising an agriculturally acceptable carrier. Where used with a feed bait composition, it is to be understood that the feed bait composition might also be applied to the plant before, after or concurrently with the chemical and/or biological agent.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component, feature or step or group of components, features or steps with or without the inclusion of a further component, feature or step or group of components, features or steps.

The invention is hereinafter described with reference to the accompanying figures and the following, non-limiting examples.

9. A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition comprising spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier, with the proviso that the feed bait composition does not further comprise a nuclear polyhedrosis virus, wherein said feed bait composition is applied before, after or together with an insecticidal and/or biological agent.

10. A method according to claim 9, wherein the one or more constituent protein(s) of said feed bait composition is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

11. A method according to claim 9 or 10, wherein the one or more constituent protein(s) of said feed bait composition is a fusolin protein.

12. A method according to claim 11, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

13. A method according to claim 9 or 10, wherein the one or more constituent protein(s) of said feed bait composition is a fusolin-like protein.

14. A method according to claim 13, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

15. A method according to any one of claims 9-14, wherein the spindle bodies, spindle-like bodies or constituent protein(s) comprise 0.05 to 15.0% (by weight) of said feed bait composition.

16. A method according to any one of claims 9-15, wherein said feed bait composition further comprises a pheromone(s) or other chemical attractive to insects.

17. A method according to any one of claims 9-16, wherein the agriculturally acceptable carrier is selected from edible substances.

18. A method of controlling or preventing damage caused to a plant according to any one of claims 1-8 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent, with the proviso that said biological agent is not a nuclear polyhedrosis virus.

19. A method according to any one of claims 9 to 18, wherein the insecticidal chemical is selected from organophosphate compounds.

20. A method according to any one of claims 9 to 19, wherein the biological agent is selected from pathogenic bacteria.

21. A method according to any one of claims 9 to 19, wherein the biological agent is selected from insect viruses other than nuclear polyhedrosis viruses.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 92368	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. **PCT/AU00/00181**	International filing date *(day/month/year)* 10 March 2000	(Earliest) Priority Date *(day/month/year)* 10 March 1999

Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readable form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.
   [ ] as suggested by the applicant.
   [ ] because the applicant failed to suggest a figure
   [ ] because this figure better characterizes the invention
   [X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00181

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A01H 5/00; A01N 63/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (WPIDS) AND CHEMICAL ABSTRACTS: KEYWORDS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
DATABASE BIOTECHABS: KEYWORDS BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, CA, BIOTECHABS. KEYWORDS: spindle body fusolin bip chaperone spindlin insect virus baculovir? entomopoxvir?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Journal of Invertebrate Pathology, vol. 71, 1998, Mitsuhashi *et al.*, "The Spindles of an Entomopoxvirus of *Coleoptera* (*Anomala cuprea*) Strongly Enhance the Infectivity of a Nucleopolyhedrovirus in *Lepidoptera* (*Bombyx mori*)", pages 186 to 188	9-22 1-22
X	Appl. Entomol. Zool., vol. 33(2), 1998, Tomita *et al.*, "Serological relationship between inclusion body proteins and a virus enhancing factor of an entomopoxvirus", pages 277 to 280	9-22 1-22
X	Journal of Invertebrate Pathology, vol. 60, 1992, Xu and Hukuhara, "Enhanced Infection of a Nuclear Polyhedrosis Virus in Larvae of the Armyworm, *Pseudaletia separata*, by a Factor in the Spheroids of an Entomopoxvirus", pages 259 to 264	9-22 1-22

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 May 2000	12 MAY 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **CHRISTOPHER LUTON** Telephone No : (02) 6283 2256

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Journal of General Virology, vol. 74, 1993, Dall *et al.*, "A gene encoding a highly expressed spindle body protein of *Heliothis armigera* entomopoxvirus", pages 1811 to 1818	1-22 1-22
X Y	Journal of Invertebrate Pathology, vol. 63, 1994, Xu and Hukuhara, "Biochemical Properties of an Enhancing Factor of an Entomopoxvirus", pages 14 to 18	9-22 1-22
X Y	Chemical Abstracts online abstract accession no. 125:295128, Kagaku to Seibutsu, vol. 34(9), 1996, Hayakawa and Takahiko, "Glycoprotein promoting infection by insect virus" pages 562 to 564	1-22 1-22
Y	Gene, vol. 177, 1996, Hayakawa *et al.*, "Cloning and sequencing of the gene for an enhancing factor from *Pseudaletia separata* entomopoxvirus", pages 269 to 270	1-22
Y	Virology, vol. 208, 1995, Gauthier *et al.*, "The *Melolontha melolontha* Entomopoxvirus (*Mm*EPV) Fusolin Is Related to the Fusolins of Lepidopteran EPVs and to the 37K Baculovirus Glycoprotein" pages 427 to 436	1-22
Y	WO 93/25666 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 23 December 1993	1-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00181

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	93/25666	AU	42973/93	BR	9306558	CN	1083527
		EP	646172	NZ	252931	US	5762924
		ZA	9304279				

END OF ANNEX

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7]: A01H 5/00, A01N 63/02

A1

(11) International Publication Number: **WO 00/53000**

(43) International Publication Date: 14 September 2000 (14.09.00)

(21) International Application Number: PCT/AU00/00181

(22) International Filing Date: 10 March 2000 (10.03.00)

(30) Priority Data:
PP 9113 10 March 1999 (10.03.99) AU

(71) Applicant *(for all designated States except US)*: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DALL, David, James [AU/AU]; 6 Cadell Street, Downer, ACT 2062 (AU).

(74) Agent: FB RICE & CO.; P.O. Box 668, Carlton South, VIC 3053 (AU).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

(57) Abstract

Plants are disclosed which are capable of expressing, in a tissue or tissues susceptible to damage by feeding insects, an exogenous protein(s) such as fusolin or a fusolin–like protein, to reduce damage to the plant by inhibiting feeding, growth and/or development of insects. Feed baits comprising spindle bodies, spindle–like bodies or constituent protein(s) thereof which inhibit feeding, growth and/or development of insects are also disclosed.

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PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

Field of the Invention:

The present invention relates to the problem of damage caused to plants (e.g. crop plants) from feeding insects such as lepidopterans and coleopterans. More particularly, the present invention relates to a plant capable of expressing, in a tissue or tissues susceptible to damage by feeding insects, an exogenous protein(s) which may reduce damage to the plant by inhibiting feeding, growth and/or development of insects.

Background of the Invention:

Entomopoxviruses (EPVs) are insect-specific members of the family *Poxviridae* (Murphy *et al.*, 1995) that collectively infect hosts such as caterpillars, beetles and locusts (Arif, 1995). Like other members of the poxvirus family (i.e., the chordopoxviruses; ChPVs), EPVs have large double-stranded DNA genomes, produce complex virions, and replicate in the cytoplasm of infected cells (Moss, 1996). While these and other molecular characteristics confirm their poxvirus affinities (Osborne *et al.*, 1996), other notable traits differentiate EPVs from ChPVs, and ally them instead with unrelated groups of insect-infecting viruses. Foremost among these traits is production of the distinctive proteinaceous structures known as spheroids and spindle bodies.

Spheroids develop in the cytoplasm of EPV-infected cells at the site of viral morphogenesis, and when mature, occlude large numbers of infectious virions (Goodwin *et al.*, 1991). They are the agent of horizontal transmission of EPVs, and while their major constituent matrix protein (spheroidin; Hall & Moyer, 1991) has no known homologue outside the taxon, the bodies themselves are assumed to protect virions from detrimental environmental factors such as desiccation and exposure to u.v. light. In this respect they are functionally analogous to the polyhedral bodies which occlude virions of members of the baculovirus family and the cytoplasmic polyhedrosis group of reoviruses.

Most EPVs also encode and produce a protein known as fusolin, which has been shown to be the major constituent of structures known as spindle

bodies (SBs; Dall *et al.*, 1993); these structures have been described from many, but not all, members of EPV genera A and B that infect caterpillars and beetle larvae (Goodwin *et al.*, 1991). In the *Heliothis armigera* EPV (HaEPV)(Fernon *et al.*, 1995), the fusolin protein has a calculated M_r of 40132, and the mature form of the protein has an apparent size of 50K when analysed by SDS-PAGE (Dall *et al.*, 1993). The protein has been found to accumulate in vesicular structures derived from cellular endoplasmic reticulum, where it eventually aggregates and crystallises into SBs (Lai-Fook and Dall, in press). Although other proteins are known to be co-located in SBs (e.g., the ER-specific chaperone protein, BiP; Lai-Fook and Dall, in press), analysis of purified SB preparations shows that fusolin, in its monomeric and multimeric forms (Dall *et al.*, 1993), is by far the most abundant constituent.

Genes encoding homologues of the fusolin protein, in this context known variously as "gp37", "37K protein", "SLP" (spindle-like protein), etc., have also been described from a number of nuclear polyhedrosis (NPV) baculoviruses, including the *Autographa californica*, *Bombyx mori*, *Choristoneura fumiferana*, *Lymantria dispar*, *Orgyia pseudotsugata* NPVs and *Xestia c-nigrum GV* (AcMNPV, BmMNPV, CfMNPV, LdMNPV, OpMNPV and XcGV, respectively; Ayres *et al.*, 1994; Gomi *et al.*, 1999; Liu and Carstens, 1996; Kuzio *et al.*, 1999; Ahrens *et al.*, 1997; Hayakawa *et al.*, 1999). In some of these (e.g., OpNPV; Gross *et al.*, 1993), the protein has been observed within spindle-like bodies (SLBs) in the cytoplasm of infected cells. SLBs have also been observed in the cytoplasm of cells infected with other NPVs (e.g., from *Cadra cautella* NPV, Adams and Wilcox 1968; see also Adams and McClintock, 1991; Cunningham, 1971; Huger and Kreig, 1968; Smirnoff, 1970).

All members of the fusolin group of proteins, irrespective of their viral family of origin, are united by an absolute conservation of amino acid residues at a number of positions in their sequences, in particular in the N-terminal and central regions of the molecule. These conserved residues include HGX (standard one letter amino acid code, where X is an aromatic amino acid) and ARQ motifs near the N-terminal of the deduced protein sequence (Table 1), and e.g. a VRWQR (SEQ ID NO:1) sequence elsewhere within the deduced amino acid sequence (Figure 1). This conservation of sequence elements, like that of the protein's intracellular location, as previously described, suggests that all members of the group also share a

common role in the cycle of virus infection and replication, perhaps in influencing the relationship of the viruses with their hosts (Sriskantha *et al.*, 1997). Nevertheless, the function(s) of members of this group of proteins, and the SB/SLB structures that they form, remain a topic of on-going investigation.

Studies by Xu and Hukuhara (1992, 1994) suggested that a factor associated with preparations of *Pseudaletia separata* EPV (PsEPV), and subsequently identified as fusolin (Hayakawa *et al.*, 1996), was capable of enhancing the infectivity of a heterologous nuclear polyhedrosis virus (*P. unipunctata* NPV). Further studies have shown that a similar effect can be seen in transgenic rice plants in which this protein has been expressed (Hukuhara *et al.*, 1999). Similarly, the SBs of the cupreous chafer (*Anomala cuprea*) have been shown to be capable of acting in the same manner (Mitsuhashi *et al.*, 1998). The role(s) of fusolin protein in the context of homologous EPV systems has not, however, been previously subjected to detailed investigation.

Through experiments involving bioassays using SBs of *Heliothis armigera* EPV (HaEPV) and *Dermolepida albohirtum* EPV ($DaEPV_{SR}$), the present applicants have determined, unexpectedly, that consumption of spindle bodies alone can effect feeding, growth and development of insect larvae. Further, through experiments conducted using recombinant EPVs wherein the fusolin gene has been replaced with a β-galactosidase marker (i.e., to render the recombinant EPVs fusolin negative [$fus^{(-)}$]), the present applicants have also been able to provide evidence to show that it is the fusolin protein component of SBs that is responsible for these effects. Moreover, the latter experiments have indicated that fusolin enhances the infectivity of the homologous EPV virus. As a result, it has been realised that SBs, SLBs and constituent proteins of these structures may be advantageously used in strategies designed to reduce damage caused to plants by feeding insects.

Summary of the Invention:

In a first aspect, the present invention provides a plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding one or more constituent protein(s) of spindle bodies or spindle-like bodies from an insect virus, operably linked to a suitable

promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

In a second aspect, the present invention provides a feed bait composition comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

Detailed disclosure of the Invention:

As mentioned above, the invention provides a plant capable of expressing one or more constituent SB/SLB protein(s) in tissues (e.g. leaf tissue or a product tissue such as fruit tissue) susceptible to damage by feeding insects. Thus, when feeding insects feed on a plant according to the invention, they will ingest, along with plant tissue, the expressed constituent SB/SLB protein(s). Since SBs/SLBs appear to inhibit feeding, growth and/or development of insects and, potentially, increase susceptibility to infection from insect pathogens (and thereby insect death), ingestion of one or more of the constituent SB/SLB protein(s) by feeding insects may reduce further damage to the plant. In addition, it is believed that inhibiting the feeding, growth and/or development of insects also increases the likelihood of insect death resulting from, for example, adverse environmental conditions, predators and chemical and other biological agents (e.g. pathogenic bacteria).

The plant according to the invention may be any plant of agricultural, arboricultural, horticultural or ornamental value that is susceptible to damage by feeding insects. Preferably, the plant is selected from plants of agricultural value such as cereals (e.g. wheat and barley), vegetable plants (e.g. tomato and potato) and fruit trees (e.g. citrus trees and apples). Other preferred plants include tobacco and cotton.

The polynucleotide molecule(s) comprising a nucleotide sequence encoding one or more constituent SB/SLB protein(s) operably linked to a suitable promoter sequence(s), may be any polynucleotide molecule(s) that may be stably segregated and retained in daughter cells. Preferably, the polynucleotide molecule(s) is stably integrated into a non-essential site within the plant genome (as may be achieved by the well known technique of homologous recombination).

Preferred constituent SB/SLB proteins are fusolins, fusolin-like proteins and the ER-specific chaperone BiP proteins and homologues thereof.

Preferred fusolin proteins include those from HaEPV, *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV (Stone River isolate; $DaEPV_{SR}$; Dall *et al*, unpublished). Most preferred is the fusolin from HaEPV such as is described in the present applicant's Australian Patent No. 668734, the disclosure of which is to be regarded as incorporated herein by reference.

The term "fusolin-like protein" refers to all insect virus proteins and functional fragments thereof which are capable of inhibiting feeding, growth and/or development in at least one insect species, and which preferably also increases susceptibility in at least one insect species to infection from at least one pathogen virus (e.g. a virus). As such, the term includes all proteins (and functional fragments thereof) from entomopoxviruses (EPVs), nuclear polyhedrosis (NPV) and granulosis (GV) baculoviruses, and all other insect viruses, that demonstrate ≥ 35% amino acid sequence identity (as calculated by the GCG Gap algorithm; Devereux *et al.*, 1984) to the HaEPV fusolin protein and which include the following partial amino acid sequences: HGX (standard one letter amino acid code, where X is an aromatic residue), and ARQ motifs near the N-terminal, and VRWQR (SEQ ID NO:1) elsewhere. Preferred fusolin-like proteins include those from AcMNPV, BmMNPV, CfMNPV, LdMNPV, OpMNPV and XcGV.

Where the plant expresses more than one constituent SB/SLB protein, the plant may be transformed with a single polynucleotide molecule such that the proteins are expressed from single or multicistronic messenger RNA. Alternatively, the proteins might be expressed from two or more polynucleotide molecules co-transformed into the plant.

Where the plant expresses all of the constituent SB/SLB protein(s) of an insect virus, the protein(s) may be present in the plant tissues in the form of SB/SLB structures.

Suitable promoter sequence(s) for the expression of the nucleotide sequence(s) encoding the constituent SB/SLB protein(s), may be selected from any promoter sequence which is functional in plants. Preferred promoter sequences include those from plants, plant viruses and plant viroids. Particularly preferred promoter sequences include the cauliflower

mosaic virus (CaMV 35S) promoter element, and promoter elements from the sub-clover stunt virus (SCSV).

Plants according to the present invention may also express an exogenous toxin or other exogenous agent that is deleterious to insects. For example, the plant may also express a *Bacillus thuringiensis* δ-toxin, an insect neurohormone, or an antisense RNA or ribozyme targeted against an essential cellular function. The heterologous toxin or deleterious agent may be encoded by a nucleotide sequence (operably linked to a suitable promoter sequence) borne on the polynucleotide molecule(s) encoding the one or more constituent SB/SLB protein(s) or may be borne on a further polynucleotide molecule which has been co-transformed into the plant.

Transformation of the plant with the polynucleotide molecule(s) may be achieved by any of the methods well known in the art including *Agrobacterium* transformation and electroporation.

As will be appreciated, the benefits achieved by expressing one or more constituent SB/SLB protein(s) in plants might also be achieved by producing feed baits comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies. Thus, feed bait compositions according to the present invention comprise spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

The feed bait compositions may be in a liquid or gel form, but more preferably are in a solid form. The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) may comprise 0.05 to 15.0% (by weight) of the composition. In addition to the spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) and the agriculturally acceptable carrier, the feed bait composition may further comprise a pheromone(s) or other chemical attractant to insects. For liquid formulations the agriculturally acceptable carrier may be selected from ingredients such as milled clays or edible carrier substances such as plant materials, molasses or raw sugar, and microorganisms such as yeasts or other fungi, algae or bacteria. For solid feed bait compositions, the agriculturally acceptable carrier may be selected from ground or fragmented plant material and other materials as described above processed to an appropriate form. The solid feed bait compositions may be provided as pellets and applied by casting over an area containing a

plant for which protection against damage by feeding insects is desired. Liquid or gelled feed bait compositions may be applied to a plant by spraying.

The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) included in the feed bait composition may be isolated from natural sources or, more conveniently, produced recombinantly in, for example, bacteria, yeast, insect or mammalian cell cultures.

Insects having ingested spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) as the result of having fed on a plant or feed bait composition according to the present invention may, as mentioned above, be expected to cause reduced damage to plants either as a result of reduced feeding/growth and/or to have reduced life times as a result of an increased susceptibility to adverse environmental conditions or chemical and biological agents. Accordingly, the present invention further extends to methods where a plant in accordance with the first aspect or a plant to which a feed bait composition in accordance with the second aspect has been applied, is treated with an insecticidal chemical and/or biological agent, and especially one whose activity has been shown to be higher against smaller, as compared to larger, insect larvae. Suitable chemical agents include organophosphate compounds and suitable biological agents include pathogenic bacteria and insect viruses (especially *Bacillus thuringiensis* [Bt] and nuclear polyhedrosis baculoviruses). These agents may be applied by any of the methods well known in the art and, most conveniently, by spraying. Preferably, the chemical or biological agent is applied in the form of a composition comprising an agriculturally acceptable carrier. Where used with a feed bait composition, it is to be understood that the feed bait composition might also be applied to the plant before, after or concurrently with the chemical and/or biological agent.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component, feature or step or group of components, features or steps with or without the inclusion of a further component, feature or step or group of components, features or steps.

The invention is hereinafter described with reference to the accompanying figures and the following, non-limiting examples.

Brief Description of the accompanying figures:

Figure 1: Provides a comparison of a partial amino acid sequence of the fusolin protein of *Dermolepida albohirtum* entomopoxvirus (Stone River isolate; $DaEPV_{SR}$) with corresponding regions of the same protein from other selected entompoxviruses and baculoviruses. Boxed text shows $DaEPV_{SR}$ fusolin sequence as determined by N-terminal amino acid analysis (bold) or conceptual translation of coding nucleotide sequence. Asterisks above the boxed $DaEPV_{SR}$ sequence show residues that differ from others of beetle-derived EPVs (MmEPV and AcEPV); those below the alignment show residues conserved across conceptual proteins from EPVs, NPVs and Gvs. (MmEPV: *Melolontha melolontha* EPV; AcEPV: *Anomala cuprea* EPV; CbEPV: *Choristoneura biennnis* EPV; HaEPV: *Heliothis armigera* EPV; BmNPV: *Bombyx mori* nuclear polyhedrosis virus [NPV]; CfNPV: *Choristoneura fumiferanae* NPV; XcGV: *Xestia c-nigrum* granulosis virus).

Figure 2: Provides a reproduction of a Coomassie blue stained SDS-PAGE gel of fractionated spindle bodies from HaEPV and $DaEPV_{SR}$.

Figure 3: Provides a map for the transfer vector pEPAS3.

Figure 4: Shows protein constituents of wild-type and recombinant [$fus^{(-)}$] isolates of IIaEPV, visualised (a) by staining with Coomassie Blue, or (b) by Western blotting with antiserum to HaEPV fusolin. Arrows indicate positions of fusolin protein.

Figure 5: Infectivity of wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV for 48 hr old *Helicoverpa armigera* larvae.

Figure 6: Shows weight gain profiles of 48 hr old *Helicoverpa armigera* larvae after 7 days feeding on diet contaminated with wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV.

Figure 7: Shows the developmental fate of 48 hr old *Helicoverpa armigera* larvae after 21 days feeding on diet contaminated with wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV.

Example 1:

Separation techniques for purification/isolation of HaEPV and $DaEPV_{SR}$ viruses and spindle bodies.

Preparations of spheroids and spindle bodies (SBs) of *Heliothis armigera* entomopoxvirus (HaEPV) and *Dermolepida albohirtum* entomopoxvirus ($DaEPV_{SR}$) were made from macerated cadavers of larvae of *Spodoptera litura* (Lep: Noctuidae) and *Dermolepida albohirtum* (Col: Melolonthinae), respectively, using a process of repeated differential centrifugation. These preparations were layered onto a 36% (w/w) solution of CsCl and spun overnight at 27000 rpm in a Beckman SW41 rotor. Fractions containing high numbers of spindle bodies were collected and pooled, and the process was repeated until a sufficient degree of purity was obtained. Purified preparations of SBs were washed three times in phosphate-buffered saline (PBS), then stored at 5°C in the same solution until use.

Preparations were analysed by light microscopy (LM) and by examination of protein composition by SDS-PAGE, the latter using techniques previously described (Dall *et al.*, 1993). These protocols showed that a very high level of purity could be achieved for HaEPV (Figure 2, lane 2), and that a satisfactory degree of purity could be obtained for $DaEPV_{SR}$ (Figure 2, lane 4).

Partial characterisation of $DaEPV_{SR}$ fusolin

Protein constituents of preparations of $DaEPV_{SR}$ were separated by SDS-PAGE and immobilised by western blotting onto PVDF membrane (Dall *et al.*, 1993). A band corresponding to a protein of about 50K M_r, and thus representing the putative $DaEPV_{SR}$ fusolin protein, was isolated, and the N-terminal amino acid sequence of the immobilised protein was obtained by use of an Applied Biosystems Procise Sequencer.

The resultant amino acid sequence (HGYITFPIARQRR (SEQ ID NO: 2); standard one letter code) was compared with others in GenBank using the NCBI Blast algorithm (Altschul *et al.*, 1990). This and other analyses (using the GCG Gap algorithm) showed that this sequence corresponded to those known from fusolin/gp37 proteins from baculoviruses (nuclear polyhedrosis and granulosis viruses; NPVs and GVs, respectively), and other EPVs, and that it most closely matched forms of the protein previously identified from

EPV isolates from coleopteran hosts (viz., *Melolontha melolontha* EPV [GenBank accession X77616], with which it was identical, and *Anomala cuprea* EPV [AB000780]; Table 1).

Table 1: Alignment of the N-terminal amino acid sequence of $DaEPV_{SR}$ fusolin with other selected sequences.

Virus	Sequence	Sequence Listing No.	GenBank Accession
Dermolepida albohirtum EPV (Stone River isolate)	HGYITFPIARQRR	SEQ ID NO: 2	
Melolontha melolontha EPV	HGYITFPIARQRR	SEQ ID NO: 2	(X77616)
Anomala cuprea EPV	HGYVTFPIARQRR	SEQ ID NO: 3	(AB000780)
Choristoneura biennis EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(M34140)
Heliothis armigera EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(L08077)
Pseudaletia separata EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(BAA09138)
Bombyx mori NPV	HGYLSLPTARQYK	SEQ ID NO: 5	(U55071)
Choristoneura fumiferana NPV	HGYLSVPVARQYK	SEQ ID NO: 6	(U26734)
Mamestra brassica NPV	HGYLSYPVARQYK	SEQ ID NO: 7	(AF108960)
Xestia c-nigrum GV	HGFMLYPLARQYR	SEQ ID NO: 8	(AF162221)
conserved residues	**....*.***..		

Genomic DNA of $DaEPV_{SR}$ was prepared by dissolution of purified preparations of spheroids/SBs in a high pH carbonate buffer containing 40mM thioglycollic acid. After the dissolution of spheroids/SBs was essentially complete (as assessed by LM examination), the solution was neutralised by addition of 10 mM Tris buffer, pH8.0, digested with protease K for 3hr, boiled for 10 minutes then centrifuged at 15K g for 10 minutes to remove residual debris. The viral genomic DNA was collected with the supernatant and stored at -20°C. Viral genomic DNA was used as template in polymerase chain reaction (PCR) protocols with custom oligonucleotide primers (oligos).

A segment of the $DaEPV_{SR}$ fusolin-encoding gene was amplified by use of custom oligos NFUS1 and EPSP6.

Oligo NFUS1 was designed by reverse translation of the $DaEPV_{SR}$ N-terminal amino acid sequence described above, and comprised the sequence:

(NFUS1) 5'-cay ggw tat atr can ttt cct ata gc-3' (SEQ ID NO: 9),

where n represents any nucleotide, r = a or g, w = a or t, and y = c or t.

Oligo EPSP6 was designed to bind to a region known to be highly conserved in other forms of the gene, located some 700 nucleotides downstream of the translation initiation codon, and comprised the sequence:

(EPSP6) 5'-aca rtt rta raa wcc ttc wcc yac-3' (SEQ ID NO: 10),

where n represents any nucleotide, r = a or g, w = a or t, and y = c or t.

PCR amplifications using oligo pair NFUS1 and EPSP6 and $DaEPV_{SR}$ DNA gave rise to a product of approximately 700 bp, as assessed by agarose gel electrophoresis. This product was cloned into plasmid pGem-TEasy (Promega), in order to allow characterisation of its constituent nucleotide sequence. The plasmid was replicated in *Escherichia coli* strain DH10β, and purified with a commercial reagent/protocol (Wizard Prep; Promega).

Analysis of the amplified nucleotide sequence used universal forward and reverse oligo nucleotides, with Elmer Perkin "Big Dye" reaction mix and PCR cycle sequence methodology as recommended by that supplier. Products of the sequencing reaction were analysed on an ABA377 DNA sequencer. The DNA sequence obtained was analysed using the GCG Map and Translate algorithms (Devereux *et al.*, 1984); related sequences were obtained from GenBank using the NCBI Blast algorithm.

Comparative sequence analyses used GCG Gap and PileUp algorithms (Devereux et al., 1984). As shown in Table 1, the available $DaEPV_{SR}$ fusolin amino acid sequence shows closest relationships to analogous regions (as construed by alignment from the N-termini of the mature forms) of fusolin proteins from EPVs of coleopteran hosts (MmEPV and AcEPV), but also shows significant levels of sequence identity to other fusolin and gp37 proteins from EPVs and baculoviruses, respectively, from lepidopteran hosts.

Alignment of conceptual amino acid sequences (Figure 1) shows that $DaEPV_{SR}$ fusolin has a unique sequence (as indicated by asterisks above the line, which show positions that differ with respect to other sequences of coleopteran EPV origin), but also that it retains the same groupings of conserved residues found in related proteins from other EPVs and baculoviruses from coleopteran and lepidopteran origins (asterisks below the alignment). As shown in Table 2, percentage identities between selected fusolin sequences range from 38.0 to 89.7% for HaEPV (complete molecule) and 45.2 to 81.8 % for $DaEPV_{SR}$ (corresponding regions).

Table 2: Relationships of deduced HaEPV and $DaEPV_{SR}$ fusolin proteins with homologues from other entomopox- and baculovirus sources.

			% identity	% similarity
[a]HaEPV	/ PsEPV		89.7	92.9
	/ CbEPV		62.9	72.6
	/ AcEPV		55.5	71.3
	/ MmEPV		52.3	67.6
	/ MbNPV		46.3	61.2
	/ BmNPV		42.8	61.6
	/ XcGV		41.7	63.6
	/ CfNPV		38.0	58.8
[b]$DaEPV_{SR}$	/ MmEPV	(19-238)	81.8	87.3
	/ AcEPV	(17-236)	72.7	81.4
	/ CbEPV	(21-241)	60.5	75.0
	/ HaEPV	(21-240)	63.6	78.2
	/ BmNPV	(20-236)	47.7	62.0
	/ CfNPV	(20-236)	45.2	61.8
	/ XcGV	(19-225)	47.0	66.8

Identities and GenBank accession numbers of viruses/sequences shown in Table 2 are as presented in Table 1.

[a] Comparison of HaEPV fusolin with other viral homologues employs deduced full length protein sequences, using the GCG Gap algorithm at default gap weight and penalty settings.

[b] Comparison of $DaEPV_{SR}$ fusolin with other homologues employs the deduced partial DaEPV sequence as presented in Figure 1, and the corresponding regions of other fusolin homologues, also as shown in Figure 1. Those regions of the latter proteins are identified in Table 2 (in brackets) by their amino acid residue numbers in their respective full length sequences. Comparisons used the GCG Gap algorithm as described above.

Bioassay of spindle body constituents against caterpillars

Purified SBs of HaEPV and $DaEPV_{SR}$ were incorporated into artificial insect diet by addition and mixing when the preparation was at a temperature just above solidification point. Diet was then allowed to solidify, and was administered to neonate larvae of *Helicoverpa armigera* and *Spodoptera litura*. Larvae were individually housed, and were reared in darkness at a constant temperature of 28°C. Larval weights and developmental status were assessed periodically; resultant pupae were stored at 5°C prior to examination (see below).

Care was taken to exclude any contribution of contaminant virus infection to outcomes of experiments. In the case of experiments using HaEPV, weights and development times of individual larvae were included in analyses only when (1) the individual larva successfully pupated and showed a normal pupal morphology, and (2) the resultant pupa was judged not to be infected by virus, as assessed by examination of tissue by light microscopy. Thus, in these experiments every individual pupa was examined before inclusion of associated data into analyses. In the case of experiments using $DaEPV_{SR}$, previous work has shown that neither caterpillar species used in bioassays here is susceptible to infection with this beetle-derived pathogen. Nevertheless, as above, data were only included in analyses in cases where individual larvae successfully pupated and showed a normal pupal morphology; in these experiments however, only pupae from larvae exposed to the highest dosage of $DaEPV_{SR}$ spindle bodies in any given experiment were assessed for the presence of virus. This methodology was designed to firstly ensure the validity of earlier studies, as noted above, and, secondly, to preclude the possibility of accidental contamination of larvae or experimental inoculum with viruses from other sources. No instance of $DaEPV_{SR}$ replication was observed.

Experiment A (#05-90526)

The experiment aimed to determine whether consumption of EPV SBs and associated fusolin protein would result in reduced rates of growth of *Helicoverpa armigera* caterpillars. Accordingly, neonate larvae of *H. armigera* were exposed to three dose rates of fusolin in SBs of HaEPV and $DaEPV_{SR}$ (dosages of 5, 50 and 100 μg fusolin/cc diet), and were assessed as described above. Seven days after commencement of the experiment, weights of subsequently "qualifying" larvae (see above) were as shown in Table 3 below:

Table 3: Weights of *Helicoverpa armigera* larvae after consumption of diet containing EPV spindle body constituents for seven days.

treatment	dose (µg/cc diet)	sample size	mean wt (gm)	standard error
control	none	23	0.0714	0.0097
HaEPV	5	27	0.0521	0.0083
HaEPV	50	21	0.0543	0.0094
HaEPV	100	16	0.0233	0.0107
$DaEPV_{SR}$	5	45	0.0642	0.0071
$DaEPV_{SR}$	50	48	0.0525	0.0069
$DaEPV_{SR}$	100	44	0.0591	0.0072

Examination of data by analysis of variance (ANOVA) showed no difference between mean weights of larvae in the control group and those fed preparations of $DaEPV_{SR}$ SBs ($P = 0.2285$), but showed that after seven days' exposure, larvae fed HaEPV SBs were significantly smaller than those in the control group ($P = 0.0201$). Analysis of larval response to different dosages of HaEPV SBs failed ($P = 0.0632$) to show evidence of a significant relationship at a 5% confidence level.

These data indicate that short term exposure of *H. armigera* larvae to the constituents of HaEPV SBs can lead to significant reductions in growth of the animal.

Experiment B (#07-90630)

The experiment aimed to determine whether consumption of $DaEPV_{SR}$ SBs would affect growth of *Helicoverpa armigera* larvae if continued for a more lengthy period, or whether consumption for an initial seven day period (as previously tested in Experiment A) would have an observable effect after a longer period of development. Accordingly, neonate larvae of *Helicoverpa armigera* were exposed to three dosages of $DaEPV_{SR}$ fusolin in SBs, and then assessed as described above. After seven days of feeding, larvae were weighed, and for each dosage regime, one sub-group was then allowed to feed on normal diet ("7d exp/ 7d normal"), while the other continued to feed on a diet containing SBs ("14d exp"). After 7 days, no significant difference was observed between mean weights of control larvae and those exposed to

$DaEPV_{SR}$ SBs (P= 0.7791); this result is consistent with that reported from Experiment A above. After 14 days, all larvae were reweighed, with results as shown in Table 4 below.

Table 4: Weights of *Helicoverpa armigera* larvae after consumption of diet containing EPV spindle body constituents for seven days (with subsequent seven days feeding on regular diet), or continuously for 14 days.

treatment	fusolin dose (µg/cc diet)	sample size	mean wt (gm)	standard error
control	none	9	0.4605	0.0389
7d exp/ 7d normal	5	16	0.4313	0.0348
14d exp	5	11	0.2194	0.0176
7d exp/ 7d normal	50	17	0.4325	0.0337
14d exp	50	10	0.2812	0.0185
7d exp/ 7d normal	100	15	0.3850	0.0359
14d exp	100	9	0.2821	0.0195

Examination of data by analysis of variance (ANOVA) showed no difference between the mean weights of larvae in the control group and those fed preparations of $DaEPV_{SR}$ SBs for 7 days prior to subsequent feeding for a further 7 days on normal diet (P = 0.3857). In contrast, highly significant differences were apparent between the mean weights of larvae in the control group and those continuously fed preparations of $DaEPV_{SR}$ SBs for 14 days (P = 0.0000), and between mean weights of the "7 day exposure/7 day normal diet" and "14 day continuous exposure" groups (P= 0.0000).

This experiment thus indicates that short term exposure of *H. armigera* larvae to the constituents of $DaEPV_{SR}$ SBs has neither short-term nor longer-term consequences, but that continuous exposure for longer periods (e.g. 14 days) causes highly significant reduction in growth. Taken across all $DaEPV_{SR}$ dosages, mean caterpillar weight was 0.2588 gm after 14 days exposure, as compared to a mean weight of 0.4605 gm for unexposed animals, representing a reduction in growth of 44%.

Experiment C (#14-91007)

The purpose of this experiment was to determine whether consumption of either HaEPV or $DaEPV_{SR}$ SBs and associated fusolin protein would affect growth of *Spodoptera litura* caterpillars, either after a 7 day period of exposure, a 7 day exposure followed by 5 days' access to normal diet, or continuous exposure for a 12 day period. Accordingly, neonate larvae of *S. litura* were exposed to one dose of SBs and associated fusolin of HaEPV (5 µg fusolin/cc diet), and to two dose rates of $DaEPV_{SR}$ SBs and associated fusolin (5 and 50 µg fusolin/cc diet).

After seven days of feeding larvae were weighed, and for each dosage regime, one sub-group was then allowed to feed on normal diet ("7d exp/5d normal"), while the other continued to feed on diet containing SBs ("12d exp"). After 7 days' feeding activity, no significant differences were observed between mean weights of control larvae and those exposed to various dosages of SBs/fusolin. After a total of 12 days feeding, larvae were reweighed, with results as shown in Table 5 below.

Table 5: Weights of *Spodoptera litura* larvae after consumption of diet containing EPV spindle body constituents for seven days (with subsequent five days feeding on regular diet), or continuously for 12 days.

treatment	fusolin identity and dose (µg/cc diet)	sample size	mean wt (gm)	standard error
control	none	25	0.7213	0.0464
7d exp/ 5d normal	HaEPV; 5	14	0.7174	0.0557
12d exp	HaEPV; 5	11	0.1719	0.0629
7d exp/ 5d normal	$DaEPV_{SR}$; 5	21	0.6829	0.0509
12d exp	$DaEPV_{SR}$; 5	17	0.1750	0.0566
7d exp/ 5d normal	$DaEPV_{SR}$; 50	16	0.5415	0.0509
12d exp	$DaEPV_{SR}$; 50	15	0.1520	0.0526

Examination of data by analysis of variance (ANOVA) showed no difference between the mean weights of larvae in the control group and those fed preparations of either HaEPV or $DaEPV_{SR}$ SBs for 7 days prior to subsequent feeding for a further 5 days on normal diet (P = 0.2973). In contrast, highly significant differences were apparent between the mean weights of larvae in the control group and those continuously fed preparations of HaEPV SBs for 12 days (P = 0.0000), or $DaEPV_{SR}$ SBs for 12 days (P = 0.0000). Likewise, highly significant differences were apparent between the mean weights of larvae in the group fed HaEPV SBs for seven days only before feeding for 5 days on uncontaminated diet, and those continuously fed preparations of HaEPV SBs for 12 days (P = 0.0000). Similarly, weights for the same comparison at each dose rate of $DaEPV_{SR}$ SBs were highly significant (P = 0.0000 for both dose rates).

These data indicate that short term (i.e., up to 7 days) exposure of *S. litura* larvae to the constituents of HaEPV and $DaEPV_{SR}$ SBs has neither short-term nor longer-term consequences, but that continuous exposure for longer periods (e.g. 12 days) causes highly significant reduction in growth.

Example 2:

Preparation of fusolin negative recombinant EPV

In this example, the transfer vector pEPAS3 (Figure 3), which contains a bacterial *lacZ* gene inserted immediately upstream of the HaEPV fusolin coding sequence, in a manner that prevents expression of the latter, was used, together with wild-type HaEPV, to produce recombinant forms of HaEPV in which fusolin production was replaced by production of the β-galactosidase marker protein. Amplified stocks of that recombinant HaEPV were subsequently found to contain forms of the virus which produced neither the β-galactosidase marker nor the fusolin protein, as judged by the absence of SBs in preparations viewed by light microscopy. Two such variants (pp5 and pp7) were isolated by repeated plaque purification and subsequent re-amplification in insect cell cultures. Harvested preparations of cells infected with these viruses were then fed to larvae of the moth *Helicoverpa armigera*, establishing, in turn, infections in those insects. Infected insects were processed to recover the products of these infections for use in subsequent biological investigations, and preparations of virus stocks

known as pp7T6 and pp7S22 (or, following a second insect passage, pp7T6/5 and pp7S22/13) were ultimately selected for more detailed characterisation.

Stocks of the wild-type clonal isolate wt#2/011293 (Osborne *et al.*, 1996), which was used as the parental form for production of the original β-galactosidase expressing recombinant, were carried in parallel through plaque purification, re-amplification, and feeding to recovery from *H. armigera* insect hosts. Selected lines from these stocks (2C1 and 2D8, or 2C1/11 and 2D8/17) served as controls in the investigations described below.

Light microscopy and scanning electron microscopy was used to examine the composition and morphology of preparations of stocks pp7T6, pp7S22, 2C1 and 2D8. As expected, preparations of the wild-type viruses 2C1 and 2D8 were observed to contain both virus spheroids and SBs, while preparations of the recombinants pp7T6 and pp7S22 were observed to contain only spheroids. The spheroids of all four stocks appeared to be morphologically identical.

The molecular composition of preparations of these virus stocks was examined using the standard laboratory protocols of SDS-PAGE and Western blotting (see, for example, Sambrook *et al.*, 1989). As shown in Figure 4(a), Coomassie Blue staining of the separated protein constituents of all four preparations showed a prominent band of about 115 kDa, corresponding to the major spheroid matrix protein (spheroidin; Hall & Moyer, 1991; Sriskantha *et al.*, 1997), and numerous other less intense bands apparently common to each. Preparations of the two wild-type stocks also showed a band of protein with a mobility of about 50 kDa, (Figure 4[a], arrow) corresponding to the monomeric form of the fusolin protein (Dall *et al.*, 1993), that was not apparent in preparations of pp7T6 and pp7S22. A polyclonal antiserum to HaEPV fusolin protein (Dall *et al.*, 1993) and Western blotting protocols were then used to further characterise these virus stocks. As shown in Figure 4(b), both preparations of wild-type virus produced very prominent immuno-reactive bands at a position corresponding to a molecular weight of about 50kDa (arrow), which, as expected, were not apparent in preparations of the two fusolin negative [$fus^{(-)}$] recombinant forms.

Feeding studies with fusolin negative recombinant EPV

One wild-type isolate (2D8) and one recombinant (pp7T6) were then selected for more detailed biological characterisation. Individually housed 48 hour old *H. armigera* larvae were exposed to a range of quantities of each

of the viruses by placing them on artificial diet spread with aliquots of virus dilution series. Seven days later ("post-infection"; 7 dpi) each larvae was weighed and at 21 dpi all larvae were collected, their developmental stage was recorded, and their status with respect to viral infection (i.e. infected or uninfected) was determined by examination of fat body smears by light microscopy. In all instances, larvae that died at or before 7 dpi were excluded from the assay, while those that were dead at 21 dpi were considered to be positive (i.e. infected).

As shown in Figure 5, these experiments demonstrated that the wild-type virus isolate 2D8 was substantially more infectious than the $fus^{(-)}$ recombinant pp7T6, with the former having an estimated IC_{50} (this being the quantity of virus required to infect 50% of exposed larvae) of 0.2 spheroids/mm^2 diet (sph/mm^2), while for pp7T6 it was 35 sph/mm^2. Results of less detailed investigations with virus isolates 2C1 and pp7S22 were also consistent with these results.

Further analysis of the results has revealed, in addition, another fusolin associated phenomenon which has not previously been recognised, namely, that the presence of fusolin is associated with retardation of the rates of growth and development of exposed insect larvae. Thus, Figure 6 shows mean weights of infected insects only, taken at 7 dpi, and calculated as a proportion of the weight of uninfected larvae from the same cohort (i.e. as a % of the weight of experimental controls). As can be observed, when the results were analysed in this manner it was clear that in the presence of fusolin, larval weight gain was much reduced. This analysis thus makes allowance for the previously described observation (i.e. that the presence of fusolin enhances virus infectivity), and further shows that when intrinsic infectivity of a particular dose is used as the basis of comparison, this previously unrecognised effect of fusolin on insect growth can be observed.

Similarly, and as shown in Figure 7, when the developmental fate of those same infected insects, now pooled in three "categories" of infection rates, was assessed at 21 dpi, a much reduced proportion of larvae was observed to proceed to pupation in samples exposed to preparations of the wild type virus containing the fusolin protein.

The above examples demonstrate the feasibility of strategies designed to effect oral ingestion of constituent SB/SLB protein(s) such as fusolin by feeding insects as a means of inhibiting feeding, growth and/or development of such insects. Such strategies may therefore be of significant value with respect to limiting losses to commodity materials that result from insect feeding activity. That is, it can be appreciated that small insects cause less feeding damage to plants than do larger ones, and that retarding the growth and/or development of insects will increase the time-span during which factors such as adverse environmental conditions, predators, and/or artificially applied chemical and biological agents may effect their control. In addition, it is widely recognised that early instar (i.e., smaller) insects are intrinsically more susceptible to infection with, or the activity of, a variety of chemical and biological control agents such as the bacterium *Bacillus thuringiensis* ("Bt").

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References:

Adams JR., McClintock JT. Baculoviridae. Nuclear polyhedrosis viruses. Part 1. Nuclear polyhedrosis viruses of insects. In JR Adams, J.R. Bonami, editors: Atlas of Invertebrate Viruses, Boca Raton: CRC Press, 1991. p 87-204.

Adams JR, Wilcox TA.. Histopathology of the almond moth, *Cadra cautella*, infected with a nuclear-polyhedrosis virus. J Invertebr Pathol 1968;12:269-274.

Ahrens CH, Russell RL, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology 1997;229: 381-399.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. 1990;215:403-410.

Arif BM. Recent advances in the molecular biology of entomopoxviruses. J Gen Virol 1995;76:1-13.

Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology 1994;202:586-605.

Cunningham JC. An ultrastructural study of the development of a nuclear polyhedrosis virus of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria*. Can J Microbiol 1971;17:69-72.

Dall DJ, Sriskantha A, Vera AP, Lai-Fook J, Symonds TM. A gene encoding a highly expressed spindle body protein of *Heliothis armigera* entomopoxvirus. J Gen Virol 1993;74:1811-1818.

Devereux J. Heaberli P, Smithies O. A comprehensive set of sequence analysis programs for the VAX. Nuc Acids Res 1984;12:387-395.

Fernon CA, Vera AP, Crnov R, Lai-Fook J, Osborne RJ, Dall DJ. *In vitro* replication of *Heliothis armigera* EPV. J Invertebr Pathol 1995;66:216-223.

Gomi S, Majima K, Maeda S. Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. J Gen Virol 1999;80:1323-1337.

Goodwin RH, Milner RJ, Beaton CD. Entomopoxvirinae. In JR Adams, J.R. Bonami, editors: Atlas of Invertebrate Viruses, Boca Raton: CRC Press, 1991. p 393-434.

Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A 37-kilodalton glycoprotein from a baculovirus of *Orgyia pseudotsugata* is localized to cytoplasmic inclusion bodies. J Virol 1993;67:469-475.

Hall RL, Moyer RW. Identification, cloning, and sequencing of a fragment of *Amsacta moorei* entomopoxvirus DNA containing the spheroidin gene and three vaccinia virus-related open reading frames. J Virol 1991;65:6516-6527.

Hayakawa T, Xu J, Hukahara, T. Cloning and sequencing of the gene for an enhancing factor from *Pseudaletia separata* entomopoxvirus. Gene 1996;177:269-270.

Hayakawa T, Ko R, Okano K, Seong SI, Goto C, Maeda S. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology 1999;262:277-297.

Huger AM, Krieg A. On spindle-shaped cytoplasmic inclusions associated with a nuclear polyhedrosis of *Choristoneura murinana*. J Invertebr Pathol 1968;12:461-462.

Hukuhara T, Hayakawa T, Wijonarko A. Increased baculovirus susceptibility of armyworm larvae feeding on transgenic rice plants expressing an entomopoxvirus gene. Nature Biotechnology 1999;17:1122-1124.

Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek JM, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology 1999;253:17-34.

Lai-Fook J, Dall DJ. Spindle bodies of *Heliothis armigera* entomopoxvirus develop in structures associated with host cell endoplasmic reticulum. J Invertebr Pathol (in press).

Liu JJ, Carstens EB. Identification, molecular cloning, and transcription analysis of the *Choristoneura fumiferana* nuclear polyhedrosis virus spindle-like protein gene. Virology 1996;223: 396-400.

Mitsuhashi W, Furuta Y, Sato M. The spindles of an entomopoxvirus of *Coleoptera* (*Anomala cuprea*) strongly enhance the infectivity of a nucleopolyhedrovirus in *Lepidoptera* (*Bombyx mori*). J. Invertebr. Pathol. 1998;71:186-188.

Moss, B. Poxviridae: the viruses and their replication. In BN Fields, DM Knipe and PM Howley, editors: Fields Virology. Philadelphia, Lippincott-Raven Publishers, 1996. p 2637-2671.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., and Summers, M.D. (editors) Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses. Vienna & New York: Springer-Verlag, 1995.

Osborne RJ, Symonds TM, Sriskantha A, Lai-Fook J, Fernon CA, Dall DJ. An entomopoxvirus homologue of the vaccinia virus D13L-encoded "rifampicin resistance" protein. J Gen Virol 1996;77:839-846.

Phanis CG, Miller DP, Cassar SC, Tristem M, Thiem SM, O'Reilly DR. Identification and expression of two baculovirus gp37 genes. J Gen Virol 1999;80:1823-1831.

Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd edn. New York: Cold Spring Harbor Laboratory.

Smirnoff WA. Rhombohedral twisted crystalloids found in the cytoplasm of nuclear polyhedrosis infected larvae of the ugly-nest caterpillar. Can J Microbiol 1970;16:906-907.

Sriskantha A, Osborne RJ, Dall DJ. Mapping of the *Heliothis armigera* entomopoxvirus (HaEPV) genome, and analysis of genes encoding the HaEPV spheroidin and nucleoside triphosphate phosphohydrolase I proteins. J Gen Virol 1997;78:3115-3123.

Xu J, Hukuhara T. Enhanced infection of a nuclear polyhedrosis virus in larvae of the armyworm, *Pseudaletia separata*, by a factor in the spheroids of an entomopoxvirus. J Invertebr Pathol 1992;60:259-264.

Xu J, Hukuhara T. Biochemical properties of an enhancing factor of an entomopoxvirus. J Invertebr Pathol 1994;63:14-18.

Claims:

1. A plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding one or more constituent protein(s) of spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, said nucleotide sequence(s) being operably linked to a suitable promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

2. A plant according to claim 1, wherein the one or more constituent protein(s) is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

3. A plant according to claim 1 or 2 which expresses a fusolin protein.

4. A plant according to claim 3, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

5. A plant according to claim 1 or 2 which expresses a fusolin-like protein.

6. A plant according to claim 5, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

7. A plant according to any one of the preceding claims which further expresses an exogenous toxin or other agent that is deleterious to insects.

8. A plant according to claim 7, wherein the exogenous toxin is selected from *Bacillus thuringiensis* δ-toxin and insect neurohormones.

9. A feed bait composition comprising spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

10. A feed bait composition according to claim 9, wherein the one or more constituent protein(s) is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

11 A feed bait composition according to claim 9 or 10, wherein the one or more constituent protein(s) is a fusolin protein.

12. A feed bait composition according to claim 11, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

13. A feed bait composition according to claim 9 or 10, wherein the one or more constituent protein(s) is a fusolin-like protein.

14. A feed bait composition according to claim 13, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

15. A feed bait composition according to any one of claims 9-14, wherein the spindle bodies, spindle-like bodies or constituent protein(s) comprise 0.05 to 15.0% (by weight) of the composition.

16. A feed bait composition according to any one of claims 9-15, further comprising a pheromone(s) or other chemical attractive to insects.

17. A feed bait composition according to any one of claims 9-16, wherein the agriculturally acceptable carrier is selected from edible substances.

18. A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition according to any one of claims 9-17 before, after or together with an insecticidal chemical and/or biological agent.

19. A method of controlling or preventing damage caused to a plant according to any one of claims 1-8 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent.

20. A method according to claim 18 or 19, wherein the insecticidal chemical is selected from organophosphate compounds.

21. A method according to claim 18 or 19, wherein the biological agent is selected from pathogenic bacteria.

22. A method according to claim 18 or 19, wherein the biological agent is selected from insect viruses.

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Gly Gly Ser Thr Ser Gln Ala Ala Ser Ala Ala Gln Tyr Met Phe Gln
    50                  55                  60

Gln Asp Asn Glu Tyr Ala Ala Leu Ala Gly Pro Asn Phe Arg Asp Ile
65                  70                  75                  80

Cys Trp Ile Lys Glu Gln Val Val Pro Asp Tyr Leu Cys Ala Ala Gly
                85                  90                  95

Ala Asp Thr Trp Arg Ile Arg Pro Phe Gly Asp Lys Thr Gly Met Asp
            100                 105                 110

Ile Val Gly Ser Trp Pro Pro Thr Val Ile Pro Leu Glu Asn Asn Phe
        115                 120                 125

Val Asn Thr Ile Pro Ile Glu Leu Glu Phe Cys Pro Thr Ala Ile His
    130                 135                 140

Glu Pro Ser Tyr Phe Glu Val Tyr Val Thr Thr Pro Glu Phe Asn Val
145                 150                 155                 160

Tyr Arg Asp Lys Val Thr Trp Pro Leu Leu Glu Leu Val Phe Asn Ser
                165                 170                 175
```

Thr Val Pro Leu Val Asn Arg Arg Ala Asp Ser Leu Cys Thr Ala Asn
180 185 190

Ala Arg Val Tyr Arg Met Ile Val Pro Val Pro Tyr Arg Gln Thr Gln
195 200 205

Phe Val Ile Tyr Val Arg Trp Gln Arg Ile Asp Pro
210 215 220

<210> 14
<211> 221
<212> PRT
<213> Choristoneura biennis entomopoxvirus

<400> 14
His Gly Tyr Met Thr Phe Pro Ile Ala Arg Gln Arg Arg Cys Ser Ala
1 5 10 15

Ala Gly Gly Asn Trp Tyr Pro Val Gly Gly Gly Gly Ile Gln Asp Pro
20 25 30

Met Cys Arg Ala Ala Tyr Gln Asn Val Phe Asn Lys Val Leu Asn Ser
35 40 45

Asn Gly Gly Asp Val Ile Asp Ala Ser Glu Ala Ala Asn Tyr Met Tyr
50 55 60

Thr Gln Asp Asn Glu Tyr Ala Ala Leu Ala Gly Pro Asp Tyr Thr Asn
65 70 75 80

Ile Cys His Ile Gln Gln Arg Val Val Pro Ser Tyr Leu Cys Ala Ala
85 90 95

Gly Ala Ser Asp Trp Ser Ile Arg Pro Phe Gly Asp Lys Ser Gly Met
100 105 110

Asp Leu Pro Gly Ser Trp Thr Pro Thr Ile Ile Gln Leu Ser Asp Asn
115 120 125

Gln Gln Ser Asn Val Val Met Glu Leu Glu Phe Cys Pro Thr Ala Val
130 135 140

His Asp Pro Ser Tyr Tyr Glu Val Tyr Ile Thr Asn Pro Ser Phe Asn
145 150 155 160

Val Tyr Thr Asp Asn Val Val Trp Ala Asn Leu Asp Leu Ile Tyr Asn
165 170 175

Asn Thr Val Thr Leu Arg Pro Lys Leu Pro Glu Ser Thr Cys Ala Ala
180 185 190

Asn Ser Met Val Tyr Arg Phe Glu Val Ser Ile Pro Val Arg Pro Ser
195 200 205

Gln Phe Val Leu Tyr Val Arg Trp Gln Arg Ile Asp Pro
210 215 220

<210> 15
<211> 220
<212> PRT
<213> Helicoverpa armigera entomopoxvirus

<400> 15

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His Gly Tyr Met Thr Phe Pro Ile Ala Arg Gln Arg Arg Cys Ser Val
 1               5                  10                  15

Arg Gly Gly Gln Trp Trp Pro Pro Asn Gly Asp Gly Ile Thr Asp Thr
            20                  25                  30

Met Cys Arg Ala Ala Tyr Gln Asn Val Tyr Asn Lys Val Leu Asn Gln
        35                  40                  45

Tyr Asn Asp Pro Gln Glu Ala Ala Thr Ala Ala Gln Tyr Met Phe Gln
    50                  55                  60

Gln Asp Asn Glu Tyr Ala Ala Leu Ala Gly Pro Asp Tyr Thr Asn Leu
65                  70                  75                  80

Cys Asn Leu Gln Gln Asn Val Val Pro Asn Asn Leu Cys Ala Ala Gly
                85                  90                  95

Ala Asp Asp Trp Asp Val Val Pro Phe Gly Asp Lys Ser Gly Met Asp
            100                 105                 110

Leu Pro Gly Asn Trp Val Pro Thr Val Ile Pro Leu Asp Ser Asn His
        115                 120                 125

Gln Ser Ser Val Ala Leu Glu Leu Glu Phe Cys Pro Thr Ala Val His
    130                 135                 140

Asp Pro Ser Tyr Tyr Glu Val Tyr Ile Thr Asn Ser Gly Phe Asn Val
145                 150                 155                 160

His Thr Asp Asn Val Val Trp Gly Asn Leu Glu Leu Ile Phe Asn Asp
                165                 170                 175

Thr Val Pro Leu Arg Pro Lys Ser Ser Thr Ser Thr Cys Asn Ala Asn
            180                 185                 190

Pro Asn Val Tyr Arg Phe Thr Val Ser Ile Pro Val Arg Pro Ala Gln
        195                 200                 205

Phe Val Leu Tyr Val Arg Trp Gln Arg Ile Asp Pro
    210                 215                 220
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<210> 16
<211> 217
<212> PRT
<213> Bombyx mori nuclear polyhedrosis virus

<400> 16

```
His Gly Tyr Leu Ser Leu Pro Thr Ala Arg Gln Tyr Lys Cys Phe Lys
 1               5                  10                  15

Gly Gly Asn Phe Tyr Trp Pro His Asn Gly Asp Lys Ile Pro Asp Ala
            20                  25                  30

Ala Cys Arg Asn Ala Tyr Lys Ser Val Tyr Tyr Lys Tyr Arg Ala Leu
        35                  40                  45

Asp Leu Glu Ser Gly Ala Ala Ala Ala Thr Ala Gln Tyr Met Phe Gln
    50                  55                  60

Gln Tyr Met Glu Tyr Ala Ser Val Ala Gly Pro Asn Tyr Asp Asp Phe
65                  70                  75                  80
```

Asp Leu Ile Lys Gln Arg Val Val Pro His Thr Leu Cys Gly Ala Gly
85 90 95

Ser Asn Asp Arg Asn Ser Val Phe Gly Asp Lys Ser Gly Met Asp Glu
100 105 110

Pro Phe Asn Asn Trp Lys Pro Asn Thr Leu Tyr Leu Asn Leu Tyr Gln
115 120 125

Pro Val Tyr Arg Met Asn Val His Phe Cys Pro Thr Ala Ile His Glu
130 135 140

Pro Ser Tyr Phe Glu Val Phe Ile Thr Lys Ser Asn Trp Asp Arg Arg
145 150 155 160

Asn Pro Ile Thr Trp Asn Glu Leu Glu Tyr Ile Gly Gly Asn Asp Ser
165 170 175

Asp Leu Ile Pro Asn Pro Gly Asp Pro Leu Cys Asp Asn Ser Leu Val
180 185 190

Tyr Ser Ile Pro Val Val Ile Pro Tyr Arg Ser Asn Gln Phe Val Met
195 200 205

Tyr Val Arg Trp Gln Arg Ile Asp Pro
210 215

<210> 17
<211> 217
<212> PRT
<213> Choristoneura fumiferana nuclear polyhedrosis virus

<400> 17
His Gly Tyr Leu Ser Val Pro Val Ala Arg Gln Tyr Lys Cys Phe Arg
1 5 10 15

Asp Gly Asn Phe Trp Trp Pro Asn Asn Gly Asp Asn Ile Pro Asp Glu
20 25 30

Ala Cys Arg Asn Ala Tyr Lys Lys Val Tyr Tyr Lys Tyr Arg Ala Ile
35 40 45

Asn Val Pro Ser Gln Glu Ala Ala Ser Ala Ala Gln Tyr Met Phe Gln
50 55 60

Gln Tyr Thr Glu Tyr Ala Ala Leu Ala Gly Pro Asn Tyr Leu Asp Phe
65 70 75 80

Asp Met Val Lys Arg Asp Val Val Pro His Thr Leu Cys Gly Ala Ala
85 90 95

Ser Asn Asp Arg Ala Ala Leu Phe Gly Asp Lys Ser Gly Met Asp Glu
100 105 110

Pro Phe Tyr Asn Trp Arg Pro Asp Val Leu Tyr Met Asn Arg Tyr Gln
115 120 125

Asn Ser Tyr Pro Met Asp Val His Phe Cys Pro Thr Ala Ile His Glu
130 135 140

Pro Ser Tyr Phe Glu Val Phe Val Thr Lys Ser Thr Trp Asp Arg Arg
145 150 155 160

```
Asn Pro Ile Thr Trp Asn Glu Leu Glu Tyr Ile Gly Gly Asn Asn Ser
                165                 170                 175

Gly Leu Val Pro Asn Pro Gly Asp Pro Leu Cys Asp Ser Asn Gln Ile
            180                 185                 190

Tyr Ser Ile Pro Val Ser Val Pro Tyr Arg Ser Gly Gln Phe Val Met
        195                 200                 205

Tyr Val Arg Trp Gln Arg Ile Asp Pro
    210                 215
```

<210> 18
<211> 207
<212> PRT
<213> Xestria c-nigrum GV

<400> 18

```
His Gly Phe Met Leu Tyr Pro Leu Ala Arg Gln Tyr Arg Cys Tyr Ala
  1               5                  10                  15

Pro Gln Asp Phe Tyr Trp Pro Asp Asp Gly Ser Asn Ile Gln Asn Pro
            20                  25                  30

Ala Cys Lys Leu Ala Phe Gln His Val Tyr Arg Asn Ser Gly Ser Ala
        35                  40                  45

Ala Ala Gln Tyr Met Phe Val Gln Tyr Ala Glu Tyr Ala Ala Leu Ala
    50                  55                  60

Gly Ser Asn Tyr Asn Asp Met Gln His Ile Gln Gln Asp Val Val Pro
65                  70                  75                  80

Asn Phe Leu Cys Ser Ala Ala Ala Asp Asn Thr Ser Thr Pro Tyr Gly
                85                  90                  95

Asp Lys Ser Gly Ile Ser Leu Pro Ser Asp His Trp Gln Thr Thr Ile
            100                 105                 110

Ile Asn Asp Arg Gly His Thr Gln Leu Tyr Tyr Cys Pro Thr Val Pro
        115                 120                 125

His Asp Pro Ser Phe Phe Gln Val Phe Val Thr Lys Lys Asp Phe Asp
    130                 135                 140

Val Gly Thr Thr Ile Val Thr Trp Asn Asp Leu Glu Leu Val His Glu
145                 150                 155                 160

Gln Ser Ala Val Ile Val Pro Asn Ser Arg Thr Val Pro Asn Ser Glu
                165                 170                 175

Glu Cys Gly Ala Phe Val Tyr Ser Ile Asp Ala Thr Leu Pro Met Arg
            180                 185                 190

Ser Lys Pro Phe Val Val Phe Val Arg Trp Gln Arg Glu Asp Pro
        195                 200                 205
```

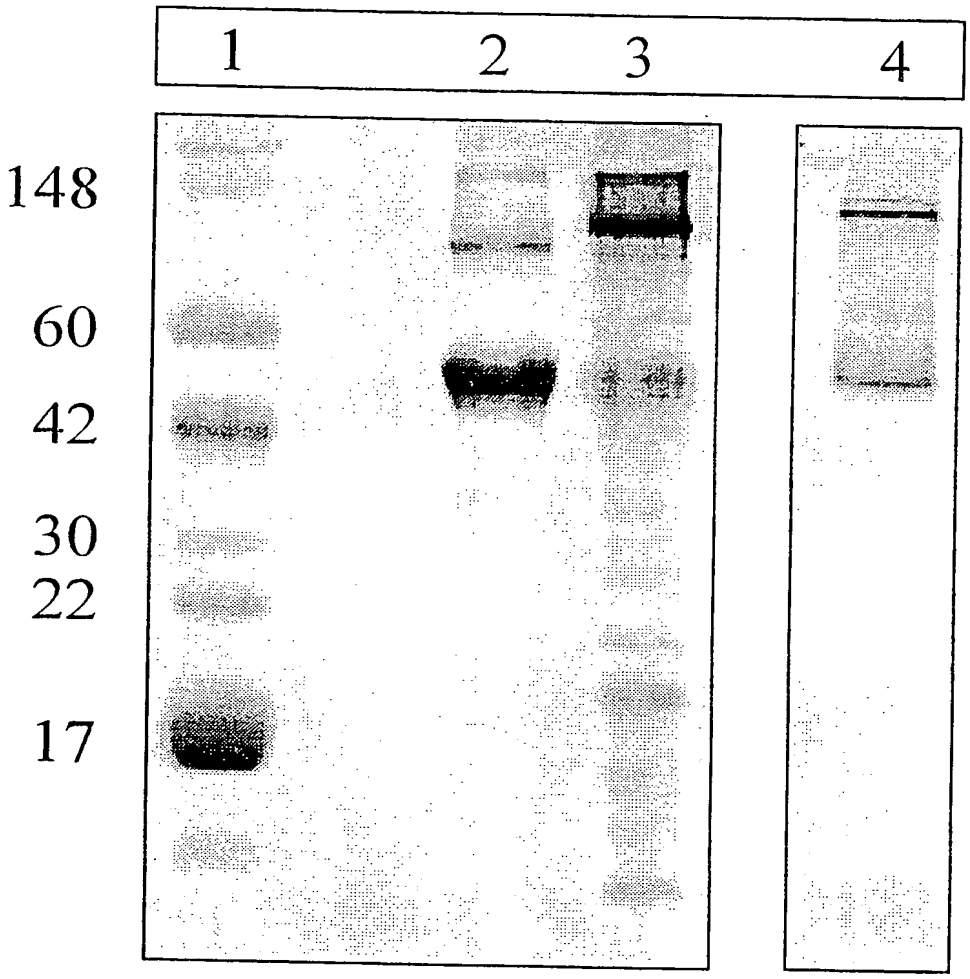
Figure 1

```
                                    **  *              *        **   *                  *
DaEPV
HGYITFPIARQRRCNVQGGFWWPTDGSAIPDPMCRAAYQNVFNTVLQQ.GGSLNQAATAAQYMFQQDNEYAALAGSNFRD
MmEPV     HGYITFPIARQRRCNVQGGFWWPPGGSGIPDPMCRAAYQNVYNKVLQQ.GGTIDQAASAAQYMFQQDNEYAALAGPNYLD
AcEPV     HGYVTFPIARQRRCNVQGGFWWPPEGTNIPDPMCRAAYQYVFNKVLSE.GGSTSQAASAAQYMFQQDNEYAALAGPNFRD
CbEPV     HGYMTFPIARQRRCSAAGGNWYPVGGGGIQDPMCRAAYQNVFNKVLNSNGGDVIDASEAANYMYTQDNEYAALAGPDYTN
HaEPV     HGYMTFPIARQRRCSVRGGQWWPPNGDGITDTMCRAAYQNVYNKVLNQY.NDPQEAATAAQYMFQQDNEYAALAGPDYTN
BmNPV     HGYLSLPTARQYKCFKGGNFYWPHNGDKIPDAACRNAYKSVYYK.YRALDLESGAAAATAQYMFQQYMEYASVAGPNYDD
CfNPV     HGYLSVPVARQYKCFRDGNFWWPNNGDNIPDEACRNAYKKVYYK.YRAINVPSQEAASAAQYMFQQYTEYAALAGPNYLD
 XcGV     HGFMLYPLARQYRCYAPQDFYWPDDGSNIQNPACKLAFQHVY.........RNSGSAAAQYMFVQYAEYAALAGSNYND
          **    * ***  *        *  *  *    *  *  *                    ***** *  **** **

          *   *     **      **  **     *   *       *    *   * *                          *
DaEPV
LNHIQNNVVPFDLCAAGANNWRRVPFGDKSGMDISGSWTPTGIPLESNTVGTGPIEFEFCPTAIHEPSFFEIYITVPNFN
MmEPV     QNHIRNNVVPNYLCAAHATTWRIRPFGDKTGMDVSGSWTPTVIPLQDNTVSTVPIEFEFCPTAIHEPSFFEIYITVPSFN
AcEPV     ICWIKEQVVPDYLCAAGADTWRIRPFGDKTGMDIVGSWPPTVIPLENNFVNTIPIELEFCPTAIHEPSYFEVYVTTPEFN
CbEPV     ICHIQQRVVPSYLCAAGASDWSIRPFGDKSGMDLPGSWTPTIIQLSDNQQSNVVMELEFCPTAVHDPSYYEVYITNPSFN
HaEPV     LCNLQQNVVPNNLCAAGADDWDVVPFGDKSGMDLPGNWVPTVIPLDSNHQSSVALELEFCPTAVHDPSYYEVYITNSGFN
BmNPV     FDLIKQRVVPHTLCGAGSNDRNS.VFGDKSGMDEPFNNWKPNTLYLNLYQPVYRMNVHFCPTAIHEPSYFEVFITKSNWD
CfNPV     FDMVKRDVVPHTLCGAASNDRAA.LFGDKSGMDEPFYNWRPDVLYMNRYQNSYPMDVHFCPTAIHEPSYFEVFVTKSTWD
 XcGV     MQHIQQDVVPNFLCSAAADNTST.PYGDKSGISLPSDHWQTTII.....NDRGHTQLYYCPTVPHDPSFFQVFVTKKDFD
                ***  ** *          *** *                          ***  * **      *

          *      *                              *    *     *
DaEPV
VFTDQVTWSQLENIFTGPIPLVARR....PDSLCNANSRVYRITVGIPMRQTQFVLYVRWQRIDP                    (SEQ ID NO: 11)
MmEPV     VYTDQVTWQQLINIFTGPIPLVQRR....PDSQCNAHNLVYRTTVGIPVRQTQFVLYVRWQRNDP          (SEQ ID NO: 12)
AcEPV     VYRDKVTWPLLELVFNSTVPLVNRR....ADSLCTANARVYRMIVPVPYRQTQFVIYVRWQRIDP          (SEQ ID NO: 13)
CbEPV     VYTDNVVWANLDLIYNNTVTLRPKL....PESTCAANSMVYRFEVSIPVRPSQFVLYVRWQRIDP          (SEQ ID NO: 14)
HaEPV     VHTDNVVWGNLELIFNDTVPLRPKS....STSTCNANPNVYRFTVSIPVRPAQFVLYVRWQRIDP          (SEQ ID NO: 15)
BmNPV     RRNPI.TWNELEYIGGNDSDLIPNP....GDPLCD.NSLVYSIPVVIPYRSNQFVMYVRWQRIDP          (SEQ ID NO: 16)
CfNPV     RRNPI.TWNELEYIGGNNSGLVPNP....GDPLCD.SNQIYSIPVSVPYRSGQFVMYVRWQRIDP          (SEQ ID NO: 17)
 XcGV     VGTTIVTWNDLELVHEQSAVIVPNSRTVPNSEEC. GAFVYSIDATLPMRSKPFVVFVRWQREDP          (SEQ ID NO: 18)
                * *                    *      *           **  ***** **
```

FIGURE 2



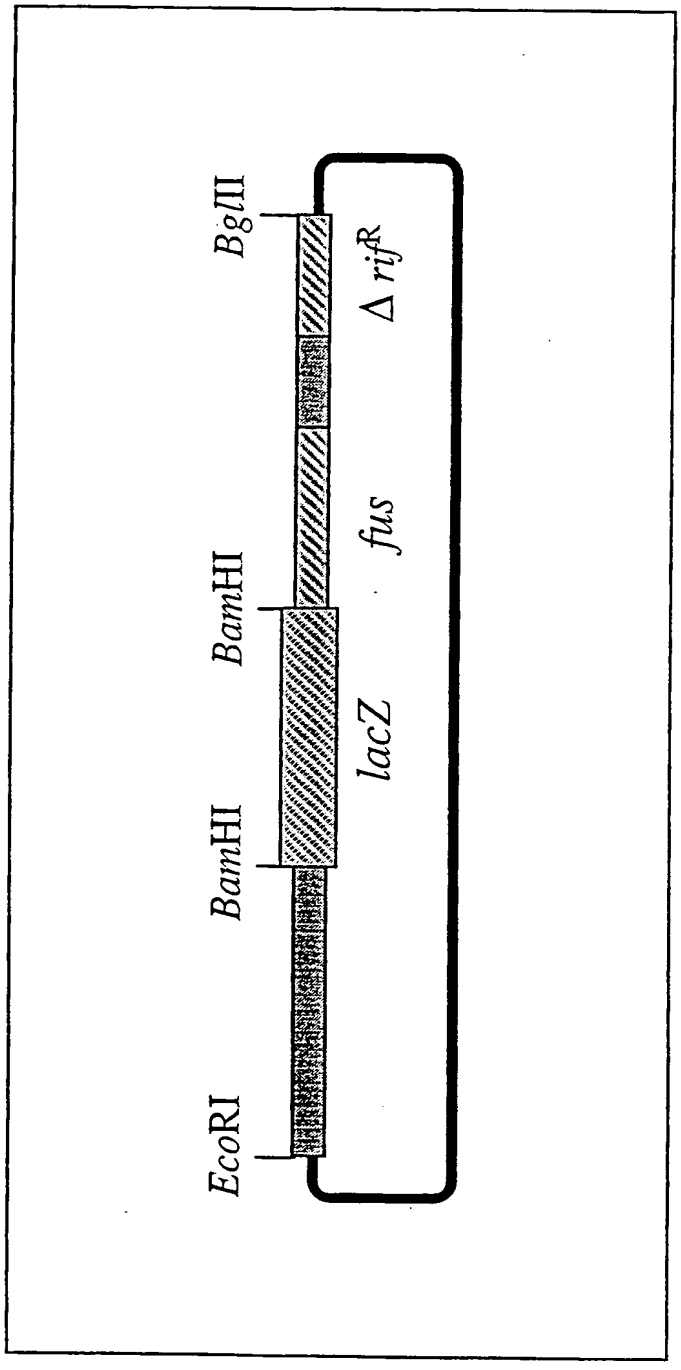


FIGURE 3

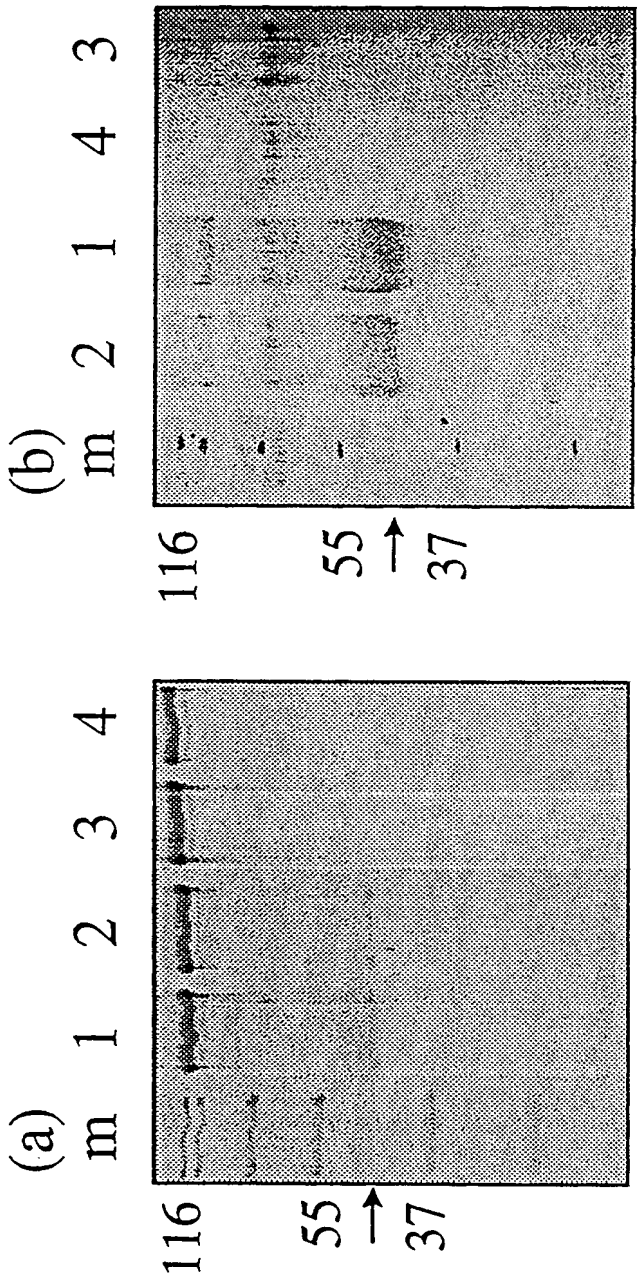


FIGURE 4

FIGURE 5

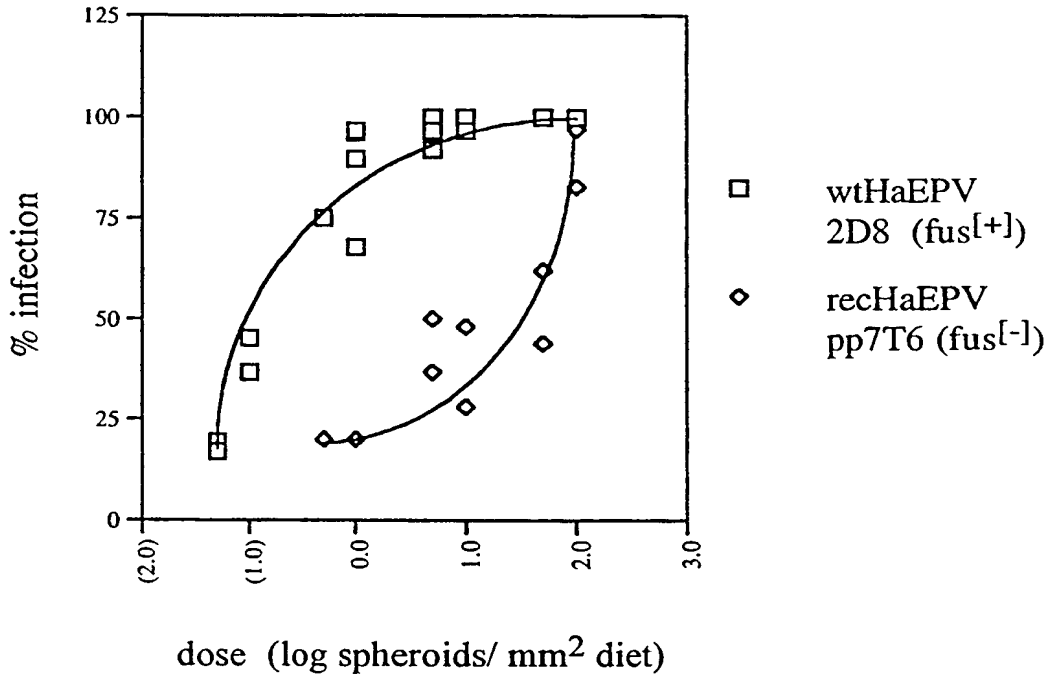
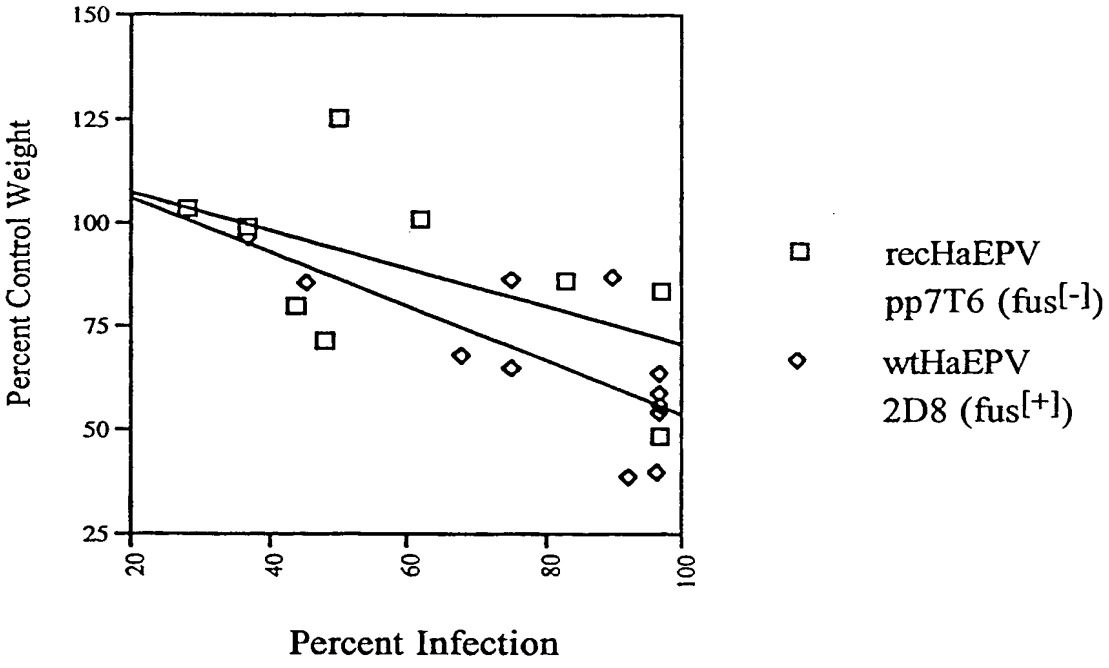

125
100
75
50
25
0
% infection
(2.0)
(1.0)
0.0
1.0
2.0
3.0
dose (log spheroids/ mm2 diet)
wtHaEPV
2D8 (fus[+])
recHaEPV
pp7T6 (fus[-])

FIGURE 6


Percent Control Weight
150
125
100
75
50
25
20
40
60
80
100
Percent Infection
recHaEPV
pp7T6 (fus[-])
wtHaEPV
2D8 (fus[+])

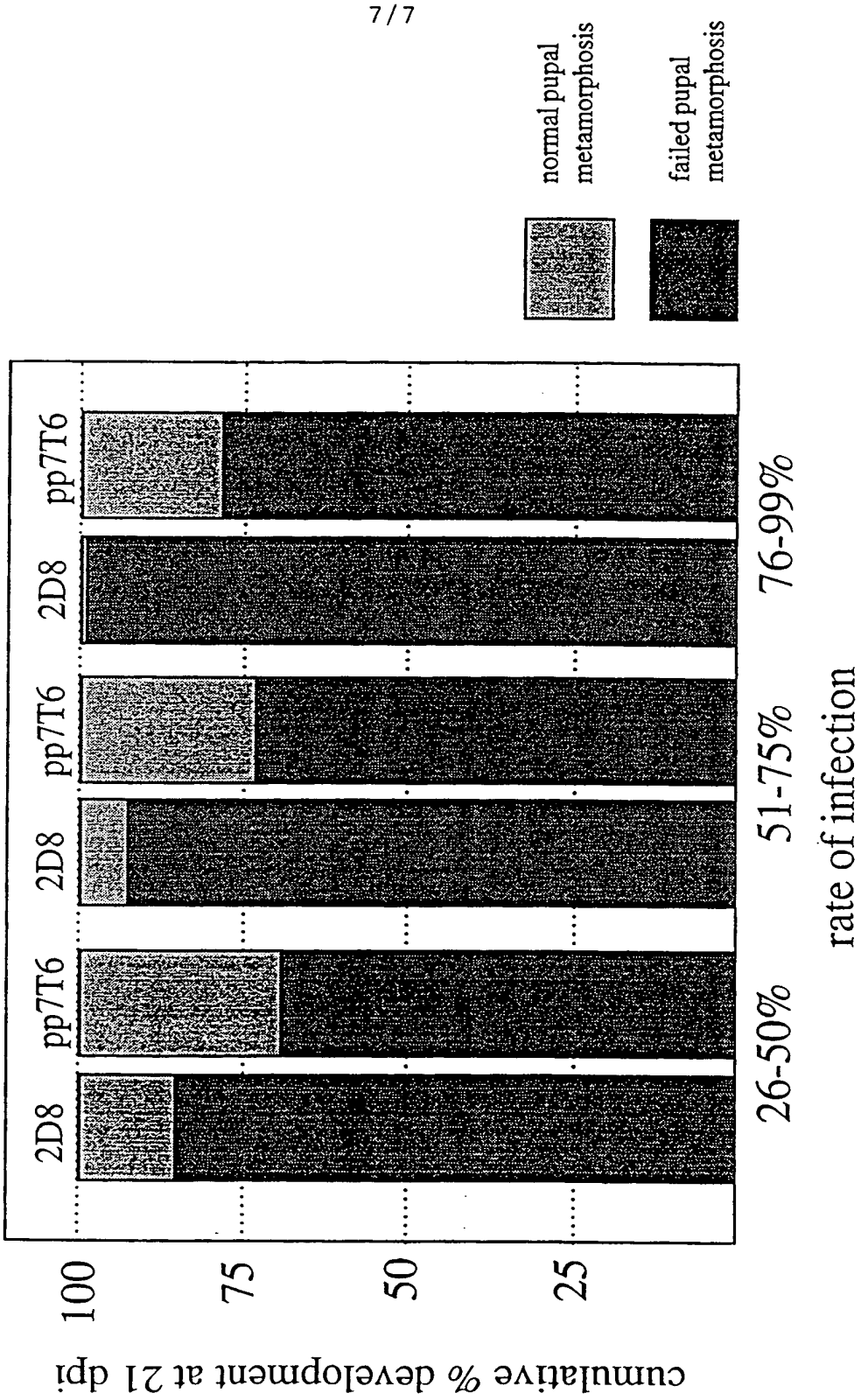

normal pupal metamorphosis
failed pupal metamorphosis
cumulative % development at 21 dpi
100
75
50
25
2D8
pp7T6
2D8
pp7T6
2D8
pp7T6
26-50%
51-75%
76-99%
rate of infection

FIGURE 7